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## IMMATURES, NATURAL HISTORY AND THE SYSTEMATIC POSITION OF *BIA ACTORION* (NYMPHALIDAE)

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**ABSTRACT.** The early stages of the Amazonian nymphalid butterfly *Bia actorion* were studied in four localities (three in Brazil and one in Ecuador). The eggs bore multiple transverse and longitudinal ridges. First instar larvae had a setose head-capsule without scoli. All other instars were characterized by basally fused, highly ramified and setose head scoli, and a bifurcate plate on the tenth abdominal segment. These characters indicate that this species (and its monotypic tribe) belong within the Brassolinae.

**Additional key words:** Areaceae, Brassolinae, life history, Satyrinae.

The genus *Bia* Hübner is monotypic, and its only species, *Bia actorion* (Linnaeus, 1763), is found throughout the Amazon from low to mid elevations in the understory of dense forests (Masters 1970). Immatures of this species were unknown, and the only studies on *Bia* were based on morphological characters of the adults (Miller 1968, Vane-Wright 1972).

There is disagreement among authors regarding the systematic position of the genus *Bia*. Weymer (1912) placed it in the Satyridae, and his classification was maintained by Ehrlich (1958), Miller (1968) and Harvey (1991); while Clark (1947, 1948) classified *Bia* in the Brassolidae, an opinion that was not widely followed. The currently accepted position of *Bia* follows Miller (1968) who included it in its own tribe Biini (subfamily Biinae, Satyridae), along with two other

tribes, Antirrhini and Melanitini. Based on a cladistic analysis of early stage characters, DeVries et al. (1985) revised Miller's (1968) classification of Biinae by transferring Antirrhini to the Morphinae. Although *Bia* was not included in their analysis, DeVries et al. (1985) noted that it possesses exposed dorsal androconial patches similar to those of *Caligo* (Brassolinae), and suggested that early stages would be informative for determining the systematic position of *Bia*.

This paper describes the immature stages of *Bia actorion* and discusses the systematic position of this species within the Nymphalidae.

### STUDY SITES

Adults and immatures of *Bia actorion* were observed and collected in the field in four different localities in the Neotropics: the DBFF project reserves north of Manaus, Amazonas, Brazil (1981); Reserva Extrativista do Alto Juruá, Upper Juruá River,

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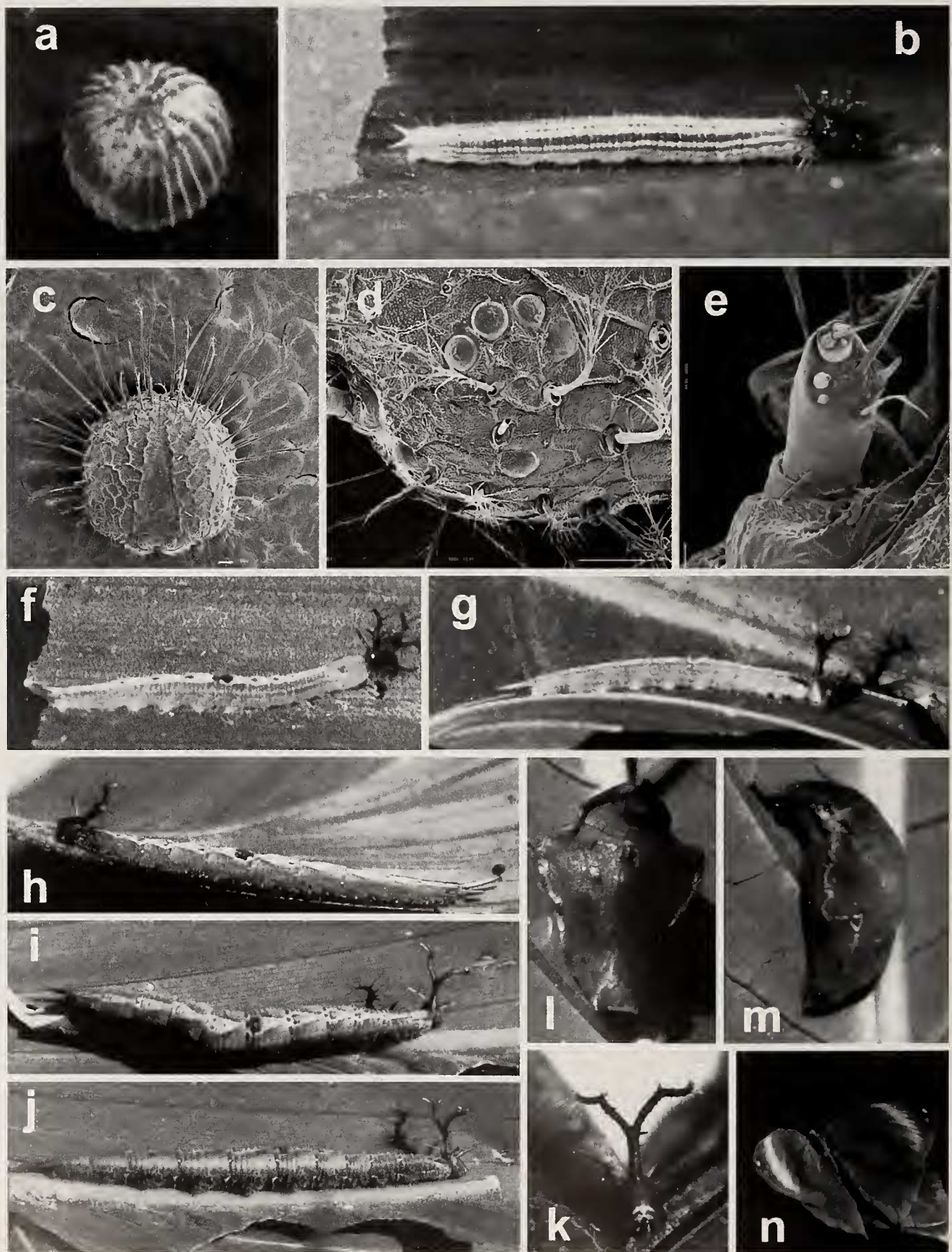
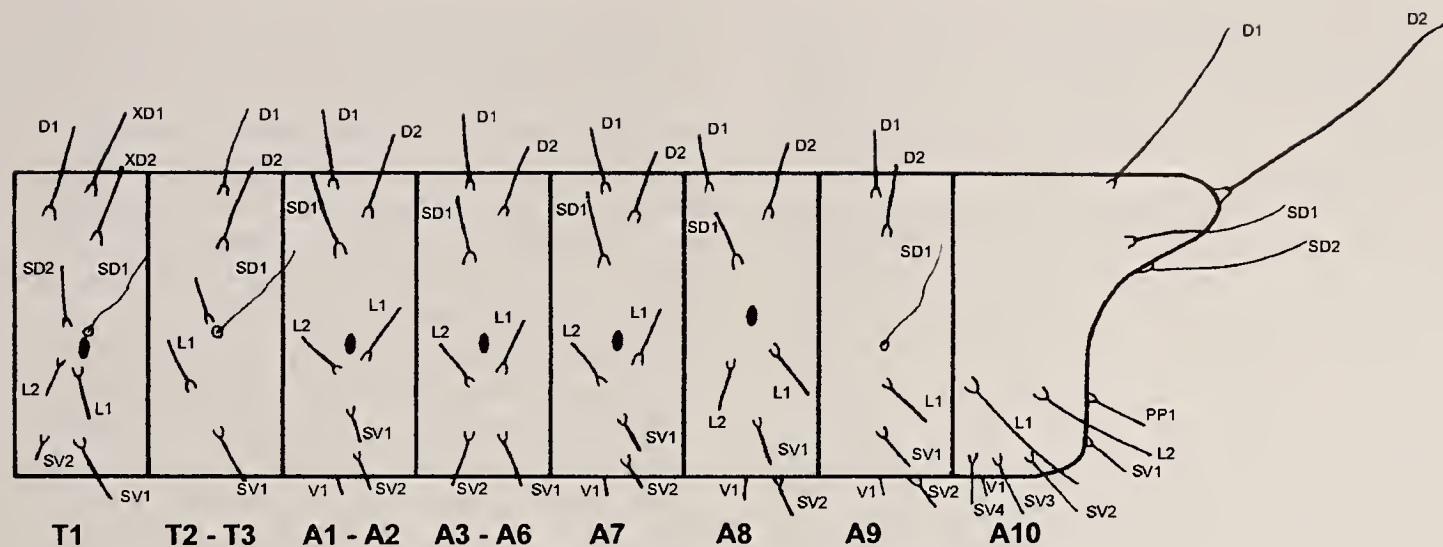


FIG. 1. Early stages and adult natural history of *Bia actorion*. **a**, egg; **b**, first instar; **c**, head capsule of first instar; **d**, lateral view of head capsule of first instar showing stemmata; **e**, antenna of first instar; **f**, second instar in molt; **g** third instar; **h**, fourth instar; **i**, **j**, fifth instar; **k**, frontal view of fifth instar head; **l**, **m**, pupa (ventral, lateral); **n**, perched adult flashing wings (dark brown, with blue and yellow on forewing).

FIG. 2. Chaetotaxy of the first instar larva of *Bia actorion*.

Marechal Thaumaturgo, Acre, Brazil (1989–1998); Jatun Sacha Biological Station, upper Napo River, eastern Ecuador (1993), and Cristalino Jungle Lodge, Rio Cristalino, Alta Floresta, northern Mato Grosso, Brazil (2000).

#### MATERIALS AND METHODS

Immatures were collected in the field in Ecuador, while in Brazil eggs were obtained from a female confined in a plastic bag with leaves of the host plant (Alta Floresta, following Freitas 1991) or through expression of the abdomen of gravid females (see Brown & Benson 1974). Immatures were reared in plastic containers, cleaned daily, and fresh plant material was provided every 2–3 days. Data were taken on behavior and development times for all stages, and head capsules and pupal castings were preserved. Immatures were fixed in Kahle (AVLF collection) or preserved in 70% ethanol after being immersed in boiling water for several seconds (DM collection). The cephalic capsule was sputter coated with gold and observed in scanning electron microscopy (Jeol JSM-5800LV). Nomenclature of body setae follows Nakanishi (1988).

#### RESULTS

**Host plants.** The host plants of *Bia actorion* were palms (Arecaceae) in all study sites. In Ecuador the host plant was the spiny palm *Astrocaryum murumuru* Martius, 1824. In Alta Floresta females were observed flying around a species of *Geonoma* palm without spines, which was used successfully as host plant. In Manaus and the Upper Juruá the palm species were not identified, but were similar to, and possibly also members of the genus *Geonoma*, used for rearing the

larva in the former case (transplanted in Monjolinho arboretum, Campinas, São Paulo, Brazil).

**Oviposition and larval behavior.** At midday on May 2, 1993, at Jatun Sacha, Ecuador, a female was observed ovipositing on a *A. murumuru* palm. Eggs were laid singly on the spines emerging from new palm fronds, or on detritus caught on the palm fronds. Oviposition was a lengthy process, with the female resting for several minutes between each egg-laying episode. Once an oviposition site was selected, the female rubbed her abdomen back and forth several times before laying eggs. The observed female laid six eggs on a single palm tree before moving off rapidly into the forest.

In Ecuador, the host plant was a tall palm tree (about 15 meters high) while in Manaus the probable host was a very small plant. In Alta Floresta and Acre, females were also observed flying around small understory palms.

Once hatched, larvae were active and moved around on the hostplant, unlike the sluggish behavior of typical satyrids (pers. obs.).

**Diagnosis for mature larva.** Head dark brown with a pale brown stripe outlining frons and a pale brown lateral stripe; dorsal head scoli fused at base, ramified and with many setae; apical and subapical setae inserted into elongated tubercles. Body slender, green, with a bright white dorsal stripe; long bifid tail present on the last abdominal segment.

**Description of early stages.** All life stages are described below based on specimens from Brazil and Ecuador.

**Egg** (Fig. 1a). Gray-white when first laid, becoming purple and pinkish-gray as the developing larva takes

form; spherical with 25–30 longitudinal ribs and up to 50 less well developed transverse ribs; height 1.2 mm, diameter 1.4 mm ( $n = 10$ , Alta Floresta). Time of development: Ecuador—9 days ( $n = 6$ ); Alta Floresta—8–10 days ( $n = 30$ ).

**First instar** (Fig. 1b–e). Head brown; setae numerous, long, black, and plumose; setae projecting anteriorly; two pairs of chalazae on vertex of head capsule. Head capsule width 0.96 mm; setae length 0.1–0.5 mm. (material from Alta Floresta, Fig. 1c). Body green, smooth, with one dorsal and three lateral red stripes, wider dorsally; T1 with a heavily sclerotized, smooth, prothoracic shield; more lightly sclerotized patch with cuticular spinules surrounding XD2 and extending dorsolaterally to posterior edge of segment; XD and D setae on T1 with bulbous apex; excepting SV and V, all abdominal setae dark, heavily sclerotized at base and with sharp spines along shaft; SD1 filiform and long on thorax and A9; L2 filiform but not long on thorax; caudal filaments well developed; D2 setae on A10 long. Body chaetotaxy illustrated in Fig. 2. Maximum length 9 mm (Alta Floresta). Duration of instar: Ecuador—11 days for 4 larvae; Alta Floresta—8 days for 30 larvae.

**Second instar** (Fig. 1f). Head dark brown dorsally and light brown ventrally and basally; two dark brown scoli at vertex of head capsule, fused into one trunk for dorsally projecting basal portion, then separating into two projecting dorsolaterally, with apex curving posterolaterally; setae on scoli plumose; two smaller pairs of dark brown subdorsal scoli on head capsule; anterior scolus projecting anteriorly and posterior scolus projecting posterolaterally, both scoli curved before apex; setae on head capsule fine and short, except setae surrounding stemmata and on base of head capsule, that are dark, thick, and plumose; microgranulations on surface of scoli and (more finely) on surface of head capsule; head capsule with weakly developed ridges in the integument. Head capsule width 1.1 mm (only 15% wider than first instar, but twice as high due to scoli); anterior scolus 0.6 mm; posterior scolus 0.8 mm; scolus on vertex: fused trunk 0.6 mm, free arms 1.1 mm (material from Alta Floresta). Body slender, dark green; dorsal median stripe bright white; red spot on A3 along midline; laterally reddish green from A8 to posterior end; caudal projections long, dark green, and projecting laterally; numerous small secondary setae on body; SD1 filiform on T1–T3, A3–A7, and A9; L2 also filiform on T1; all filiform setae with small, slightly elevated bases and light sclerotized patch. Maximum length 15 mm (Alta Floresta). Duration of instar: Ecuador—9 days ( $n = 3$ ); Alta Floresta—14 days ( $n = 25$ ).

**Third instar** (Fig. 1g). Head as in previous instar except for the following: head capsule all dark brown including basally, with pale brown stripe outlining frons and pale brown lateral stripe; setal bases surrounding stemmata and on base of head capsule larger, with setae emerging subapically; large, elongate setal bases present on apex of each scolus and several along shafts, all emerging subapically with apex smooth and pointed; integumental ridges outlining faint, shallow depressions on head capsule. Head capsule width 1.5 mm; anterior scolus 0.8 mm; posterior scolus 1.2 mm; scolus on vertex: fused trunk 1.0 mm, free arms 1.8 mm (material from Alta Floresta). Body same as in previous instar except lateral red coloration present on abdominal segments. Maximum length 30 mm (Alta Floresta). Duration of instar: Ecuador—10–11 days ( $n = 2$ ); Alta Floresta—11 days ( $n = 20$ ).

**Fourth instar** (Fig. 1h). Very similar to third instar, with the general patterns darker than in previous instars. Head capsule width 2.1 mm; anterior scolus 1.3 mm; posterior scolus 1.8 mm; scolus on vertex: fused trunk 1.8 mm, free arms 2.7 mm (material from Alta Floresta). Maximum length 35 mm (Alta Floresta). Duration of instar: Ecuador—9 days ( $n = 2$ ); Alta Floresta—11 days ( $n = 16$ ).

**Fifth instar** (Fig. 1i–k). Head same as previous instars except for the following: head capsule and scoli black frontally and brown basally, with a white stripe laterally on head capsule; scoli larger than previous instar; more secondary setal bases on scoli and around stemmata and base of head capsule forming elongate projections with subapical setae; setal bases of these setae also larger than in previous instar. Head capsule width 2.9 mm; anterior scolus 2.2 mm; posterior scolus 2.5 mm; scolus on vertex: fused trunk 2.5 mm, free arms 3.1 mm (Alta Floresta). Body slender, green; dorsal median stripe bright white; stripe narrows abruptly at anterior edge of each segment and then widens to posterior edge, with size change greatest on A1 to A6; within white stripe thin gray line along midline and reddish gray coloration, darkest on A3, lateral to gray line at widest portion of white stripe; small white dots dorsolaterally and reddish-brown coloration ventrolaterally; caudal filaments long, dark greenish-gray, projecting laterally; secondary setae on body dark brown, wide, and flattened; filiform setae on body with heavily sclerotized and small setal bases, projecting only minimally from body surface and surrounded by large, sclerotized patch. Maximum length 55 mm (Alta Floresta). Duration of instar: Ecuador—10 days ( $n = 2$ ); Alta Floresta—14 days ( $n = 13$ ).

The larvae from Ecuador passed through an additional sixth instar. The head capsule was the same as in

the previous (fifth) instar; the body was the same except for more extensive reddish-gray coloration within the white stripe, reducing white coloration to a thin outline, and white dots dorsolaterally larger. Duration of instar: 22–25 days ( $n = 2$ ).

**Pupa** (Fig. 1 l–m). Short and bumpy, slightly curved anteriorly and strongly curved at A4. Entirely green, with dark lateral keels and a shiny dot near the wing caps. Total length 15 mm (Alta Floresta). Duration of pupa: Alta Floresta—17 days ( $n = 8$ ).

**Adult behavior.** Adults were found in dense forest with abundant small palms, including gallery (riparian) forests in an upland cerrado (savannah) landscape (Vilhena, 600 m, SE Rondônia, near Mato Grosso border, Brazil).

Adults fly in the understory and are active from before dawn to nightfall, being easily found in patches with rotting fruits on which they feed. During afternoons and on cloudy days, [the] males can be found in small openings in the vegetation, often near streams, but not always near areas with palms. Through marking of individuals (DM) males were found to persist for up to 20 days. Although some individuals moved from patch to patch over time, most remained loyal to a particular area. Interactions between males were common. No mating attempts with passing females were observed, although courtship behaviors were commonly seen. Courtship appeared to be lengthy and complex, including several different tandem flight patterns, male contact with females during flight, and solo male flight over perched females while they flash their wings. Occasionally courtship behaviors were observed between two males.

#### DISCUSSION

Regarding the controversy about the classification of *Bia* (Vane-Wright 1972, DeVries et al. 1985), the early stages give additional clues about its possible systematic position. The eggs, with many transverse and longitudinal ridges, and the first instar larvae with the head capsule lacking any kind of scoli and bearing long setae, giving a furry appearance, are most similar to those of the Brassolinae (Casagrande 1979, 1992, DeVries 1987). Although some Satyrinae have eggs with ridges (Emmel & Mattoon 1972), eggs of Brassolinae are distinctive in having many well-marked ridges (30–60), considered a trait exclusive to this subfamily (Freitas 1999). The mature larva also bears a typical brassoline feature, three pairs of scoli on the head capsule. This condition is observed in most Brassolinae (except in the gregarious *Brassolis*, that lack the head projections) and is absent in all known satyrine larvae, that show head capsules bearing a single pair of scoli

on the vertex (pers. obs). Additionally, palms are common host plants of Brassolinae, whereas in Satyrinae palm feeding is rare (DeVries 1987, Ackery 1988).

Based on adult morphology, *Bia* was suggested to be more closely related to Brassolinae than to Satyrinae (Vane-Wright 1972, DeVries et al. 1985). Our examination of early stages strongly supports this hypothesis. Confirmation must await a thorough phylogenetic study and broader sampling of immatures among Satyrinae and Brassolinae.

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